CERTIFICATION OF APPROVAL

Study of Metal Ion Removal in Industrial Waste Water by Chitosan

by

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Approved by,

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CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgments, and that the original work contained herein have not been undertaken or done by unspecified sources of persons.

NURAINE BINTI IBERAHIM

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In the name of God, the Compassionate, the Merciful. The author would like to thank Him for the strength, skill, knowledge, patience and health that have been given through this time.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Currently, a process for removing heavy metals from waste water is being developed by using chitosan beads which is a selective, high capacity adsorbent. The process minimizes and generates a concentrated heavy metal stream that can be processed to recover the heavy metal. This chitosan is derived from chitin, a natural cationic biopolymer.

Crab shells are the main source of chitin because this latter can be found in high capacities. Chitin or chitosan can be also extracted from fungi or insects exoskeletons. Because of its high contents of amine and carboxyl group, chitosan is apt to form complexes with metal ions in waste water. This adsorbent is found to be chemically effective in terms of metal ion sorption.

In that case, this project is more likely to study on the metal ion removal in industrial waste water by chitosan. The source of chitosan will be obtained from crab shell since it is found as the main source of chitin. This project is specifically focusing on the metal ion removal from industrial waste water, where it has been found to be the major contribution of the current environmental issues.

Instead of that, the ease of obtaining the source of chitosan and its economical value seems to be significant to generate an effective adsorbent for metal ion removal in waste water. Metals ion such as Zinc (Zn^{2+}), Lead (Pb^{+2}) and Iron (Fe^{2+}) are selected in this project as the target ions to be removed since they are found to be poisonous heavy metal ions beside chromium (Cr^{+3}), cadmium (Cd^{+2}) and manganese (Mn^{+2}).

Therefore, this project can be beneficial for the improvement of metal ion removal from industrial waste water specifically by using extracted chitosan.

1.2 Problem Statement

In recent years, heavy metal pollution of water is pervasive and extremely serious environmental problem. The factors that contribute to this matter are mainly causes by the rapid growth of industrial development and activities. Some heavy metals such as cadmium, lead, zinc, nickel, copper and chromium (III) or their compounds have been used extensively by various industries thus leading to sharp increase in the contamination, especially in water. Because of their toxicity, the presence of any of these metals in excessive quantities will interfere with many beneficial uses of the water.

As a result, the standard discharge limit of these metals under Environmental Quality Act 1974 of Malaysia, (Sewage and Industrial Effluents) 1979 are kept below 1.0 mg/l in which the standard for iron is 1.0 mg/l, 0.10 mg/l for lead, 1.0 mg/l each for zinc, nickel, copper and chromium (III).

With regards to the environmental issues that face today, this Final Year Project has chose to study on the metal ion removal in industrial waste water by generating an effective adsorbent that capable to perform at various temperature conditions and contact time. The extracted chitosan from crab shell will be used in this study as the adsorbent to remove the metal ion present in waste water and study its efficiency towards the reduction process.

1.3 Objective and Scope of Study

With the problem statement that has been discussed earlier, the objectives that are going to be achieved in this Final Year Research Project are summarized as follows:

i. To extract chitosan from crab shell and determine on the weight percentage of the biopolymer that can be extorted.
ii. To remove metal ion such as Zn^{+2}, Pb^{+2} and Fe^{+2} from industrial waste water.
iii. To study on the metal ion removal efficiency influenced by various temperature condition and different time in contact.

The first objective of the experiment which is the extraction of chitosan from chitin is conducted by grinding and drying the crab shells to powder form. Next, chitin will be extracted from the powder by undergoing some chemical reaction which involves the deacetylation process. Then, the raw chitin is treated with 50% NaOH (w/v) weight–volume. Finally, the product is extracted with acetic acid solution.

The second objective of this final year research project is to remove selected metal ion content in industrial waste water. The chitosan extracted from the first part will be used to remove metal ions content such as Zn^{+2}, Pb^{+2} and Fe^{+2} in industrial waste water. The following concentration of the metal ions remains in industrial waste water after the treatment will be tested and analyzed.

Further study of this experiment is to analyze the metal ion removal efficiency influence by various temperature conditions and time in contact.

CHAPTER 2

LITERATURE REVIEW AND THEORY

2.1 A Brief History of Chitosan

Chitosan has a history of about three decades of use in processes like detoxifying water. When is spread over the surface of water, it literally absorbs greases, oils, heavy metals and other potentially toxic substances. Like a "fat magnet," it attracts these bio-hazardous substances from drinking water to such an extent that a scum forms in the water, which can be easily removed. Water purification plants throughout the nation such as China and United States of America (USA), use Chitosan for this purpose. This indicates to scientists that Chitosan can selectively absorb fats even in a water medium.

2.2 Chitosan

Theoretically, based on K.M Peiselt da Silva [14], chitin is the structural polysaccharide in the exoskeleton of animals. It is the polymer of n-acetylglucosamine, where a minority of the acetyl groups has been lost, generally less than 50%. Chitin is an amide of acetic acid available in large amounts from arthropods [1]. Its chemical structure can be observed in Figure 2.1. Chitin stoichiometry is $(C_8H_{13}NO_5)_n$ and contains 6.9% of nitrogen.

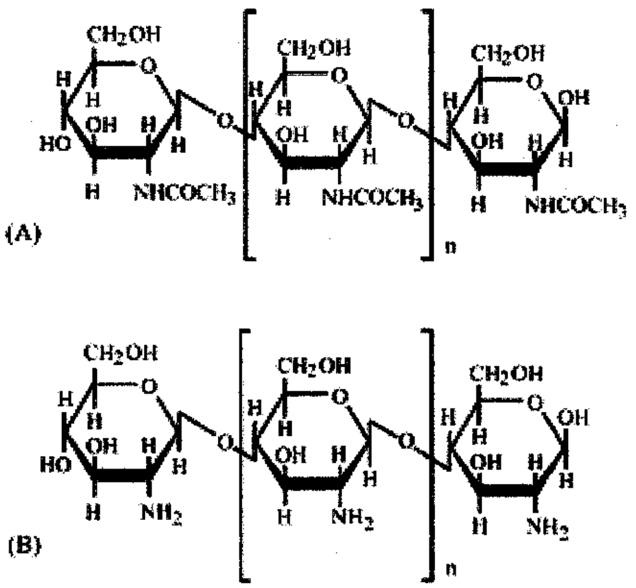


Figure 2.1: Chemical structures of biopolymers used: (A) chitin and (B) chitosan.

Chitosan, partially n-deacetylated chitin $(C_6H_{11}NO_4)_n$, is a primary amine where the amino group is free and contains 8.7% nitrogen. In chitosan, the majority of the acetyl groups have been removed generally over 70% [2]. Chitin and Chitosan are both copolymers of glucosamine and n-acetylglucosamine and may be considered as high molecular weight biopolymers.

The applicability of these chitinous materials is large considering their chemical, physical and biological properties [3 and 4]. One of the most important properties of chitin and chitosan is their ability to remove metal ions [5]. Their structure allows excellent complexation capacity with metal ions, particularly transition and post–transition metals [6]. It was supposed that the chelation of a single metal ion by several ---$NHCOCH_3$ groups effectively isolates each metal ion from its neighbors [7].

Consequently, chitin and chitosan may be used in waste water treatment for the removal of cation such as copper ions [1]. Recently, Valentini et al. [8], used chitosan – polyvinyl alcohol (PVA) capsules to remove copper an nickel in aqueous solutions. Gyliene et al. [9], used chitin and chitosan to remove free and complexed Fe, Cu, Pb, Zn, Ni and Mn from aqueous solution Benguella and Benaissa [10], studied the sorption of Cd, Cu and Zn from aqueous systems using chitin and Chu [11], removed Cu from aqueous solution with chitosan.

2.3 Flame Atomic Absorption Spectrometer (FAAS)

Flame atomic absorption is a very common technique for detecting metals and metalloids in environmental samples. It is very reliable and simple to use. Figure 2.2 shows which elements are commonly detected through atomic absorption. The technique is based on the fact that ground state metals absorb light at specific wavelengths. Metal ions in a solution are converted to atomic state by means of a flame. Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve.

With the capabilities of this equipment, the FAAS will be used in the analysis of metal ion removal by chitosan in industrial waste water.

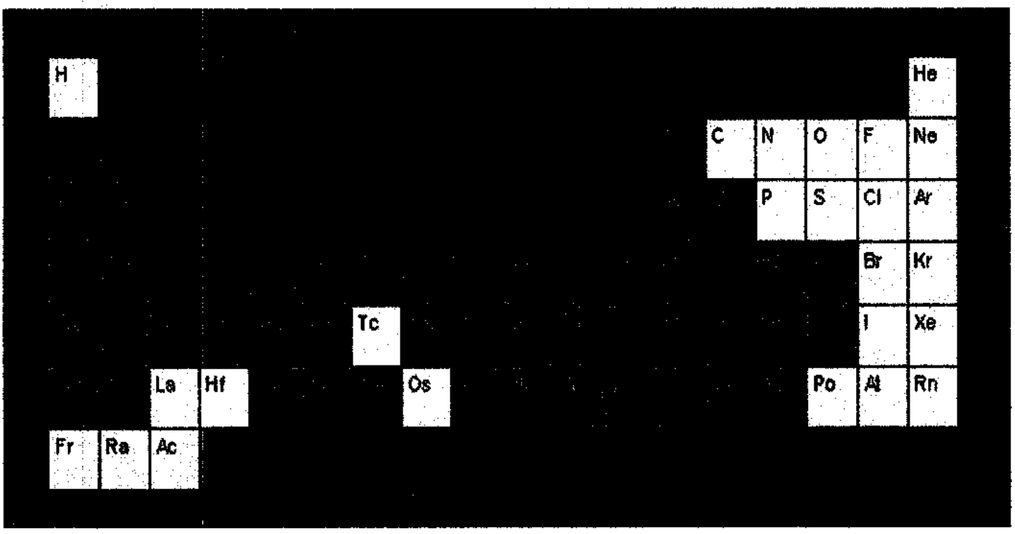


Figure 2.2: Elements detectable by atomic absorption are highlighted in pink in this periodic table.

CHAPTER 3

METHODOLOGY AND PROJECT WORK

3.1 Extraction of Chitin from Crab Shells

The methodology involves in this project are mainly on the experimental lab work, where chitosan will be extracted from crab shell by undergoing certain processes such as demineralization, deproteinization and deacetylation. The Method of Broussignac is use for demineralization and deproteinization of chitin and for deacytylation of chitin to chitosan, Method of Alimunar and Zainuddin will be applied.

3.1.1 Preparation of Crab Shells Sample

In the experiment, the chitin will be extracted from crab shells. Crab shells were purchased from the nearest market and all the inner part of it were removed and cleaned. The crab shells were then dried up under the hot sun for a few days in order to remove the unpleasant odor produced by it. Then, they were crushed into smaller pieces using pestle and mortar. The dried, smaller pieces will be ready to grind until powdery form is obtained. The specimen was then grinded using a lab grinder to make the powder finer. This was also to ensure large surface area when it reacted with the chemicals which will be added in the next procedure. The crab shell's powder were then placed in the oven at 120°C for 2 hours to make it more brittle and to prevent organic decomposition. Figure 3.1 shows the prepared crab shells sample.

3.1.2 Procedures in Preparation of Crab Shells Sample.

i. The crab shells were shattered into small pieces by using a pestle and mortar.
ii. The small pieces were then grinded into fine powder using lab grinder.
iii. The fine powder was dried in an oven at 120°C for 2 hours to dry the shells and made them more brittle.

Note: This step is also to extend the shelf life and preventing organic decomposition.

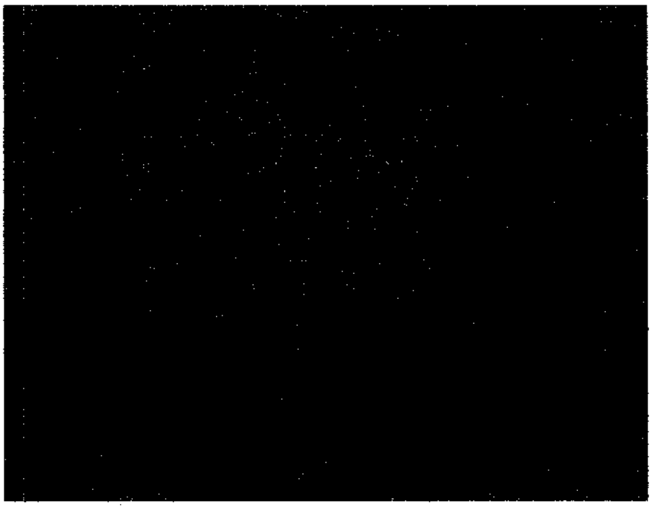


Figure 3.1: Crab shells powder.

3.1.3 Extraction of Chitin using Method of Broussignac

There were two stages involved in the extraction of chitin from crab shells. The first stage was involving the demineralization process and the second stages were the deproteination process.

Demineralization is the removal of minerals, primarily calcium carbonate ($CaCO_3$). A mineral free chitin would be required for applications that have very low impurity tolerance. This process involves the decomposition of $CaCO_3$ to calcium salts, carbon dioxide (CO_2) and water (H_2O). Hydrochloric acid (HCl) is used because it produces calcium chloride ($CaCl_2$), but the large volumes of $CaCl_2$ solution must be disposed. During the demineralization process, the aqueous solution turned to cloudy residue with formation of foams.

Deproteination is a process where covalent chemical bond has to be destroyed between the chitin–protein complexes in order to form free radicals. Free radicals are active sites of molecules which in presence will enhance the growing of molecular chains of biopolymers.

This process is achieved with some difficulties especially if performed heterogeneously utilizing chemicals that will also depolymerized the biopolymer. Sodium Hydroxide (NaOH) is the preferred reagent and typically 1.2 M of NaOH solutions is used with the variations in the temperature and duration of treatments times. Figure 3.2 shows the mixture of solution sample after the deproteination process.

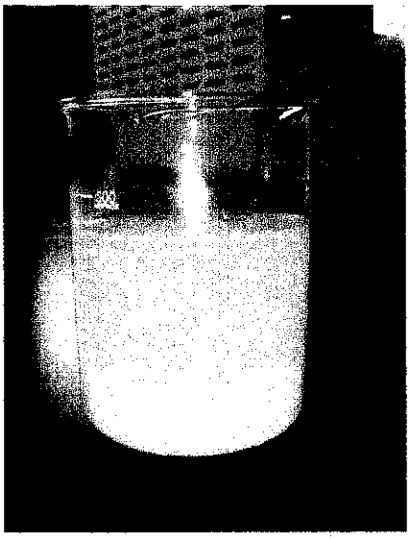
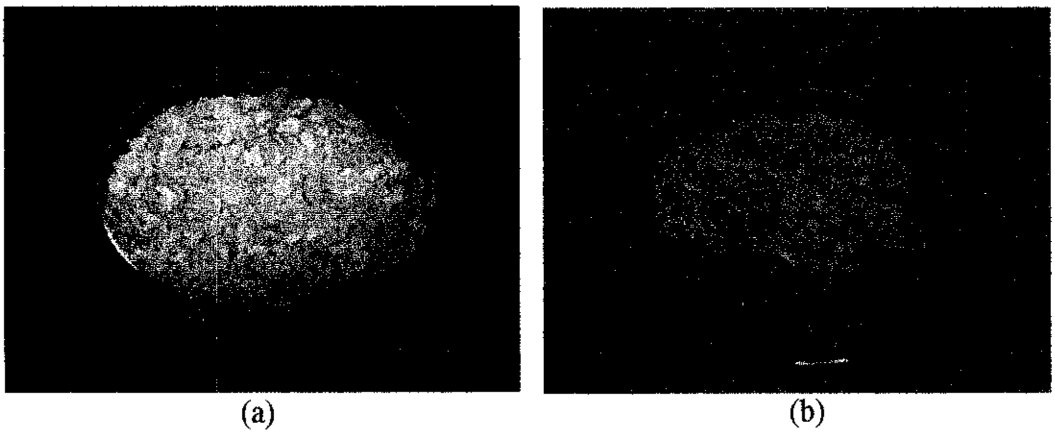


Figure 3.2: Mixture of solution sample after deproteination process.

The use of NaOH invariably results in partial deacetylation of chitin and deproteination of the biopolymer that lowers the molecular weight of chitin. The yield of sample residue at this point is yet to be determined because of the current drying condition. The powdery sample collected at this point was composed of chitin together with an intermediate amount of chitosan. Figure 3.3 shows the products collected from the preparation of crab shell sample, namely chitin and chitosan.



(a) (b)

Figure 3.3: Product of crab shell powder. a) Chitin, b) Chitosan.

The removal of these two major components, calcium carbonate and proteins gives raw reasonable quality. However, it must be noted that the quality of the final chitin is directly dependent on the starting raw material that needs to be considered. The manner in which the sample are gathered, cleaned, dried and powdered will affect the final quality of the chitosan as this determine the amount of impurities and the reactivity of the sample to the chemicals. Raw chitin can be further characterized for specific purpose based on end user requirement, i.e. in biomedical applications.

3.1.4 Procedures in Extraction of Chitin

i. 20 ml of 1.5 M of Hydrochloric acid (HCl) was added per gram of shell powder. This is to neutralize the shell powder by removing $CaCO_3$ constitute in the organic component of the shell.

ii. The mixture was kept for 2 days with constant stirring, to react to completion. Absence of effervescence was taken to mean all reaction had ceased. Most of the solution was decanted off at the end.

iii. 20 ml of 1.2 M of Sodium Hydroxide (NaOH) was added in similar proportion of HCl.

iv. The mixture was then heated at 90°C in a water bath for 1 hour.

v. Next, the mixture was left to react for 1 day at room temperature.

vi. After the reaction completed, the powdery residue was collected by using Buchner funnel, washed with distilled water and dried with acetone wash (Figure 3.4).

vii. Lastly, the sample was left overnight to let the weight stabilized. The total weight of chitin extracted was measured and recorded.

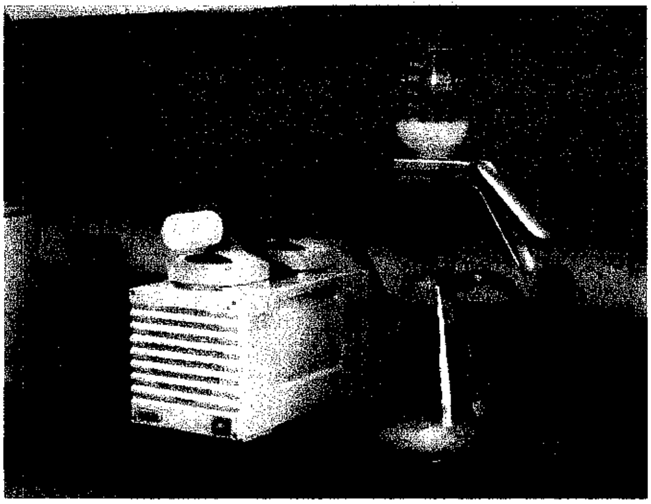


Figure 3.4: Filtration of powdery residue by using Buchner Funnel.

3.1.5 Calculation on preparation of 1.5 M HCl and 1.2 M NaOH Solutions

The 1.5 M HCl and 1.2 M NaOH solutions was prepared by adding some amount of 36% of HCl and concentrated NaOH respectively. The amount required for the preparation of the solutions can be calculated as follows:

<u>Quantity of HCl that were used in preparation of 1.5 M concentration.</u>

Let; 1 = HCl 36%

2 = HCl 1.5M

The quantity required for preparation of 1.5M solution can be calculated by using the equation (1) below:

$$M_1V_1 = M_2V_2 \text{ -- } (1)$$

Where;

Density of HCl 36%, M_1 = 1.19 kg/l (stated on HCl bottle)

Volume of pure HCl, $V_1 = x$ *(Qty of HCl required for 1.5M concentration)*

Density of 1.5M HCl, M_2 = 0.05469 kg/l (calculated)

Volume of 1.5M HCl V_2 = 500 ml = 0.5 l (Qty of 1.5 M HCl to be prepared)

Calculation of M_2

M_2 = Molarity x Molecule Weight of HCl (MWHCl)

= 1.5 mol/l x 36.46 g/mol

= (54.69 g/l)(1kg/1000g)

= 0.05469 kg/l

Thus, V_1 can be calculated:

$M_1V_1 = M_2V_2$

$V_1 = M_2V_2/M_1$

= (0.05469 kg/l)(0.5 l) / (1.19 kg/l)

= 0.002297 l

= **22.97 ml of HCl**

<u>Quantity of NaOH that were used in preparation of 1.2 M concentration.</u>

The mass of NaOH can be calculated by using the equation (2) and (3) below:

$$\mathbf{c = n/v} \quad \text{--} \quad \mathbf{(2)}$$

$$\mathbf{n = m/\ MW_{NaOH}} \quad \text{------------------------------------} \quad \mathbf{(3)}$$

Where;

Concentration of NaOH (molarity), c = 1.2 mol/l

No. of mol of NaOH = n

Mass of NaOH, required to be calculated = m

Molecular Weight of NaOH, MW_{NaOH} = 40.0 g/mol

Volume of solution to be prepared, V = 500ml = 0.5 l

Thus, the mass of NaOH is calculated as:

$c = n/v$

$n = cv$

$m/MW_{NaOH} = cv$

$m = cV \times MW_{NaOH}$

= (1.2 mol/l)(0.5 l)(40.0g/mol)

= **24.0 g of NaOH pellets.**

3.2 Chitin to Chitosan

3.2.1 Deacetylation of Chitin to Chitosan using the Method of Alimunar and Zainuddin.

At alkali concentration below 45%, chitosan will not be formed (Alimunar and Zainuddin, 1992) [15]. Higher concentrations of alkali correspond to a faster rate of deacetylation. 50 ml of concentrated solution, 50% w/v (weight – volume) NaOH 12.5M, was mixed with each gram of chitin.

50% w/v NaOH is prepared by mixing 50 g NaOH in 100 ml distilled water. As the concentration is too high, the procedures need to be done carefully. The solution prepared was kept in the fume hood as safety precaution step.

After left for complete deacetylation process, the solution was diluted before filtration. The previous step was done before filtration to prevent the concentrated solution from dehydrating the filter paper. The residue of the mixture was noticeably pale in color, as compared to the previous yield of chitin.

3.2.2 Procedure in Deacetylation of Chitin

i. 50% w/v NaOH was prepared by mixing 50g NaOH in 100 ml distilled water.
ii. Then, the solution prepared was placed in water bath 95°C for 3 hours.
iii. 50ml of concentrated solution of 50% (w/v) NaOH was mixed with each gram of chitin.
iv. The mixture was left to react for 3 days.
v. The solution was then chilled and diluted with distilled water until the pH is neutralized.
vi. Next, the mixture was extracted with 2% acetic acid, filtered and precipitated to obtain the purified chitosan.
vii. The powdery residue was dried with acetone wash before it was collected.

The following flow diagram summaries the biopolymer preparation of chitosan:

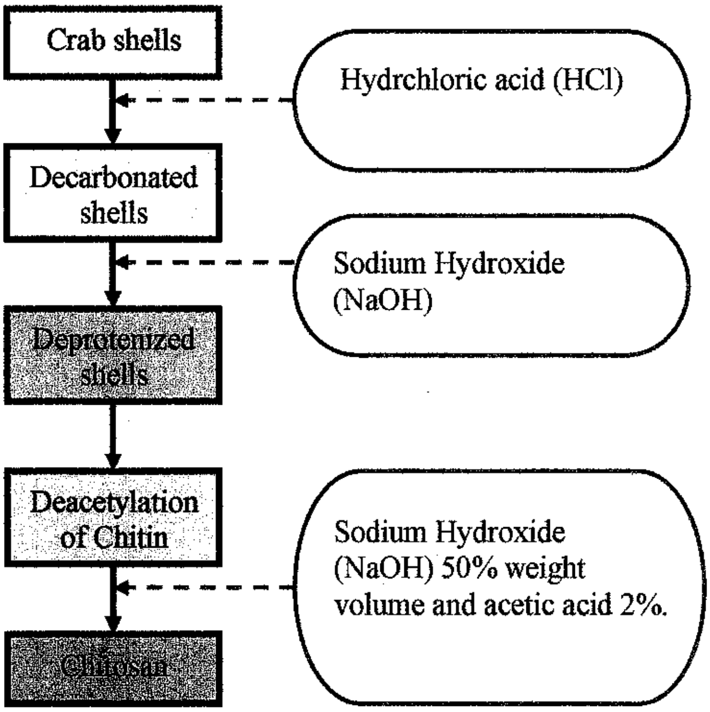


Figure 3.5: Extraction of chitosan from crab shell.

3.3 Industrial Waste Water

The industrial waste water that used in this final year research project was collected from FELCRA Nasaruddin Palm Oil Mill, which is an oil palm factory. This factory is located at Bota, Perak Darul Ridzuan, 20 km from north of Universiti Teknologi Petronas.

In general, the activity that conducted at FELCRA Nasaruddin Palm Oil Mill is mainly on the palm oil processing from the raw fruit to produce crude palm oil. The by product of the process plant are dross of the oil palm and waste water. The waste water that are produced will undergo a treatment process before it is released to the near by river.

The production processes of the crude palm oil at the factory are summarized in the simplified process flow diagram (Figure 3.6) below:

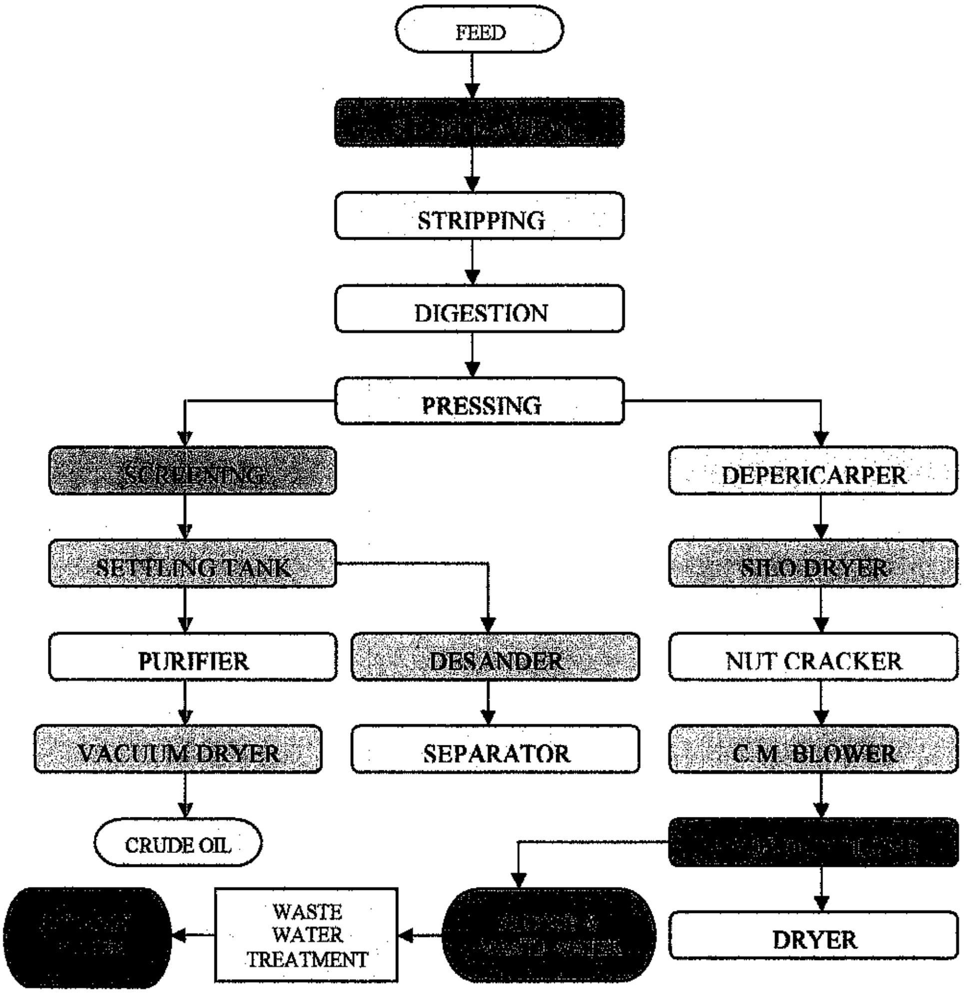


Figure 3.6: Palm Oil Processing Flow Diagram of FELCRA Nasaruddin Palm Oil Mill

Basically, the waste water of the oil palm factory was biologically treated by using BICHEM 1008 and 1003 bacteria. These types of bacteria are grown in 3 anaerobic ponds which act on reducing the number of biological oxygen demand (BOD) that contain in the water. The total amount of treated or effluent water produced from this plant is 537 810 kg per day. Therefore, it will be an advantageous for this project to study and develop new method that can be applied in the industrial waste water treatment.

The waste water samples were collected from the palm oil factory at 2 different locations. The first location of waste water sampling was collected from the final process of the hydrocyclone where sludge and water were produced. The second sampling location was collected after the waste water treatment process, where effluent water was produced. Figure 3.7 and Figure 3.8 show the locations of waste water sampling. However, the untreated industrial waste water (Figure 3.7) which basically contains sludge will be used in this project.

Figure 3.7: Sludge and waste water from waste stream

Figure 3.8: Palm Oil Mill Effluent (POME) water stream.

The waste water samples collected have been kept in the refrigerator in order to preserve from microorganism growth and to maintain the quality of the sample. But before this sample is ready to be used, it needed to undergo a filtration process in order to remove any particles and residue that might be contaminated in the waste water. The filtration process was conducted by using Buchner Funnel, the same method as the filtration of chitosan residue. This is important, in order to minimize error factor during the study of metal ion removal and to avoid any nozzles blockage while running the Flame Atomic Absorption Spectrometer (FAAS). Figure 3.9 shows the untreated industrial waste water from FELCRA Nasaruddin Palm Oil Mill and Figure 3.10 shows the industrial waste water sample that has been filtrated for the use in this research project.

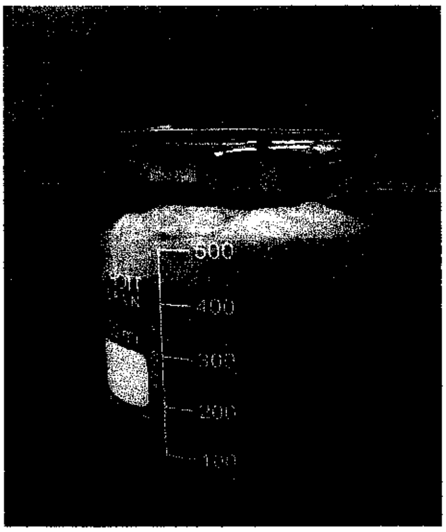


Figure 3.9: Untreated Industrial Waste Water from FELCRA Nasaruddin Palm Oil Mill.

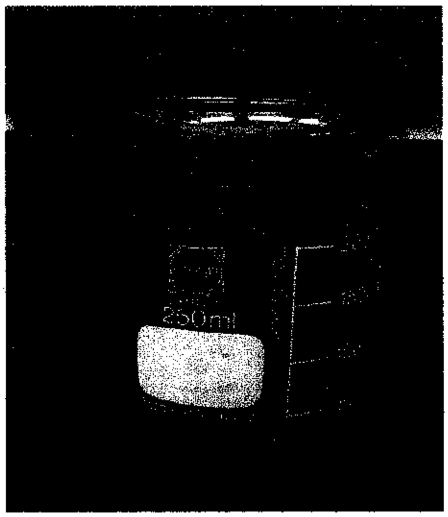


Figure 3.10: Untreated Industrial Waste Water After the Filtration Process.

3.4 Chitosan Analysis

The next progress of this research project is to study and understand the principles and techniques applied in Flame Atomic Absorption Spectrometry.

3.4.1 Flame Atomic Absorption Spectrometry

The technique of Flame Atomic Absorption Spectroscopy (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800°C.

During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths, as shown in Figure 3.11.

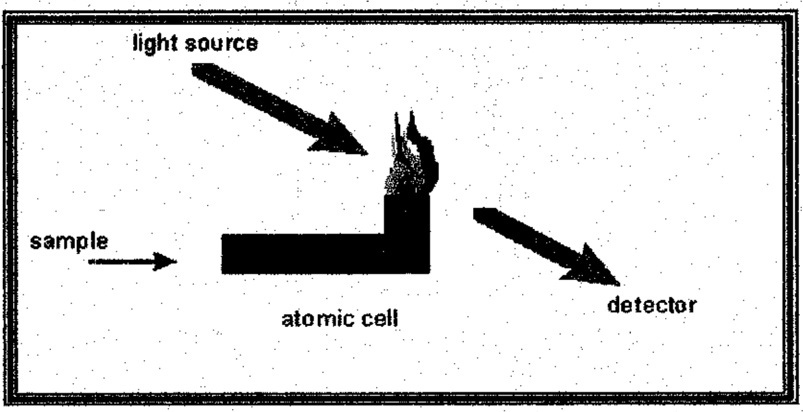


Figure 3.11: Operation principle of an atomic absorption spectrometer.

The characteristic wavelengths are element specific and accurate to 0.01-0.1nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as photonmultiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample.

3.4.2 Basic Instrumental Parts of Atomic Absorption Spectrometer

Flame atomic absorption hardware is divided into six fundamental groups that have two major functions: generating atomic signals and signal processing. Signal processing is a growing additional feature to be integrated or externally fitted to the instrument. The instrument parts are shown in Figure 3.12.

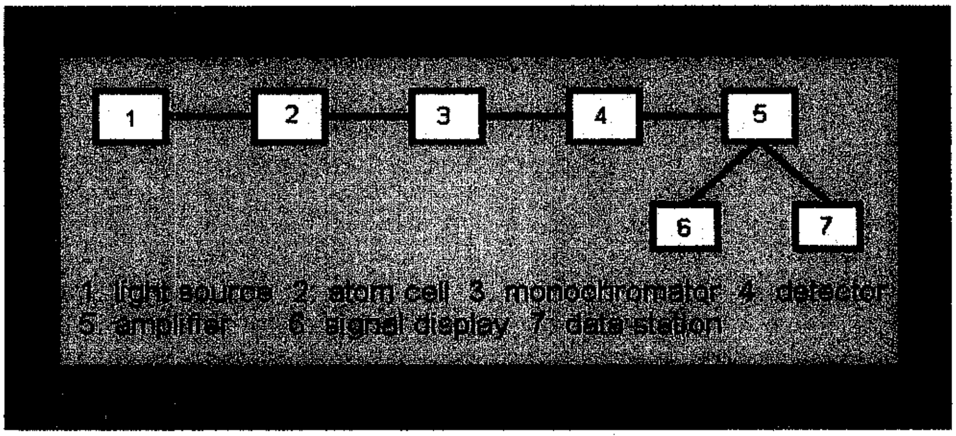


Figure 3.12: Schematic diagram of basic instrumental parts of atomic absorption spectrometer.

A cathode lamp (1), is a stable light source, which is necessary to emit the sharp characteristic spectrum of the element to be determined. A different cathode lamp is needed for each element, although there are some lamps that can be used to determine three or four different elements if the cathode contains all of them. Each time a lamp is changed, proper alignment is needed in order to get as much light as possible through the flame, where the analyte is being atomized, and into the monochromator.

The atom cell (2), is the part with two major functions: nebulization of sample solution into a fine aerosol solution, and dissociation of the analyte elements into free gaseous ground state form. Not all the analyte goes through the flame, part of it is disposed as shown in the figure.

As the sample passes through the flame, the beam of light passes through it into the monochromator (3). The monochromator isolates the specific spectrum line emitted by the light source through spectral dispersion, and focuses it upon a photomultiplier detector (4), whose function is to convert the light signal into an electrical signal.

The processing of electrical signal is fulfilled by a signal amplifier (5). The signal could be displayed for readout (6), or further fed into a data station (7) for printout by the requested format.

3.4.3 Procedures of Metal Ion Analysis for Industrial Waste Water Sample

i. The standard solution for ion metal to be tested, Zn^{2+}, Fe^{2+} and Pb^{2+}, were prepared by dilution method. The concentration of the standard solution that were used; 0.2mg/l, 0.4mg/l and 0.8mg/l for Zn^{2+}, 1.0mg/l, 2.0mg/l and 4.0mg/l for Fe^{2+}, and 4.0mg/l, 8.0mg/l and 16.0mg/l for Pb^{2+}.

Note: Deionized (DI) water was used in the preparation of the standard solution.

ii. 5 ml of the standard solutions prepared was used in the analysis of the selected metals ion presence. Each of the solution was placed in test tubes that were provided for the FAAS usage.

iii. Next, 5 ml of each waste water sample was also placed in test tubes that were provided.

iv. All of the standard solutions and samples were placed in the FASS test tubes holder and recoded accordingly.

v. The FAAS equipment then, was ran in an automatic mode to detect the selected metals ion presence.

The above procedures were repeated for the metal ion detection in the industrial waste water collected, before and after the metal ion removal process from the waste water. The initial and final amounts of the metal ion concentration were then recoded. With this data taken, the percentage of metal ion removed from industrial waste water can be determined. The results of the analysis were shown in Chapter 4 and Chapter 5.

3.5 Study of Metal Ion Removal Efficiency

The next step of this research project was to determine the optimum amount of chitosan to be used in the experiment, followed by the study on the metal ion removal efficiency influence by various temperature conditions and at different time in contact. The following procedures were used to identify the efficiency of the selected metal ion removal.

3.5.1 Determination of the Optimum Amount of Chitosan in Metal Ions Removal

i. 5 samples of 30ml industrial waste water sample were prepared in 150 ml beaker.

ii. 0.20g, 0.50g, 1.00g, 1.50g and 2.00g of chitosan were added in each of the beaker respectively.

iii. The samples were then placed under continuous stirring (speed of agitation 60 rpm) at room temperature for 12 hours of reaction.

iv. The final concentration of the metal ion that was leftover in the samples was tested by using the FAAS.

v. The weight percentage of the metal ion removed was calculated and graphs of the metals ion uptake was plotted against the amount of chitosan added.

vi. The optimum amount of chitosan used to remove the selected metals ion can be determined from the graphs plotted.

3.5.2 Efficiency of Metal Ion Removal at Different Time in Contact

i. 5 samples of 30ml industrial waste water were prepared in 150ml beakers.
ii. 2.00g of chitosan powder were added into each of the beakers.
iii. Each of the samples were placed under continuous stirring (speed of agitation 60 rpm) at room temperature for 0.30 hours, 2.00 hours, 4.00 hours, 6.00 hours and 8.00 hours respectively.
iv. The final concentration of the metal ion that was leftover in the samples was tested by using the FAAS.
v. Next, the weight percentage of the metal ion removed was calculated and graphs of the metals ion uptake was plotted against the time in contact.
vi. The trend of the metals ion reduction over period of time can be determined from the graphs plotted.

3.5.3 Efficiency of Metal Ion Removal at Various Temperatures Condition

i. 5 samples of 30ml industrial waste water were prepared in 150ml beakers.
ii. 2.00g of chitosan powder were added into each of the beakers.
iii. The samples were placed under continuous stirring (speed of agitation 60 rpm) for 0.30 hours at room temperature, 40°C, 50°C, 60°C and 70°C.
iv. The final concentration of the metal ion that was leftover in the samples was tested by using the FAAS.
v. Next, the weight percentage of the metal ion removed was calculated and graphs of the metals ion uptake was plotted against the temperature conditions.
vi. The trend of the metals ion reduction over the temperature conditions can be determined from the graphs plotted.

3.6 Tools

Tools that are required for conducting the extraction and analysis of heavy metal ion removal by chitosan are:

i. Flame Atomic Absorption Spectrometer (FAAS)
ii. Buchner Funnel
iii. Microsoft Excell

CHAPTER 4
RESULTS

4.1 Extraction of Chitosan

The extraction of chitin and chitosan from 100g crab shell powder gives the final product of 30.32g chitosan (Table 4.1). Please refer to *Appendix 3* for raw data of the experiment.

Table 4.1: Total weight and weight percentage of chitosan obtained through out the extraction processes.

Stages	Crab Shell Powder	Extraction of Chitin from Crab Shell Powder	Extraction of Chitosan from Chitin
Weight (g)	100	44.62	30.32
Wt %	-	44.62	67.95

4.2 Concentration of Heavy Metal Ion in Industrial Waste Water

The heavy metal ion detected from FELCRA Nasaruddin Palm Oil Mill waste water sample contain iron, zinc and lead, chromium, copper and nickel as shown in Table 4.2.

Table 4.2: The initial concentration of selected metals ion present in waste water sample from FELCRA Nasaruddin Palm Oil Mill.

[illegible]	[illegible]	[illegible]
Fe	24.56	2.54
Zn	2.83	0.00
Pb	0.23	0.10
Cr	0.07	0.01
Cu	0.23	0.06
Ni	0.00	0.00

4.3 Metal Ion Removed by Chitosan

The results for three heavy metal ion namely Zn^{2+}, Fe^{2+} and Pb^{2+} removed by chitosan as shown in Table 4.3, Table 4.4 and Table 4.5 accordingly.

Table 4.3: Results of Zn^{2+} ion uptake at various amount of chitosan added.

Sample	mass of chitosan added (g)	Zn^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.20	2.54	2.28	2.41	1.00
2	0.50	2.58	2.46	2.52	1.00
3	1.00	2.50	2.40	2.45	1.00
4	1.50	2.69	2.55	2.62	1.00
5	2.00	2.74	2.59	2.67	1.00

Table 4.4: Results of Fe^{2+} ion uptake at various amount of chitosan added.

Sample	mass of chitosan added (g)	Fe^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.20	10.12	9.62	9.87	1.00
2	0.50	11.44	11.30	11.37	1.00
3	1.00	13.08	12.87	12.98	1.00
4	1.50	14.07	14.01	14.04	1.00
5	2.00	17.18	17.11	17.15	1.00

Table 4.5: Results of Pb^{2+} ion uptake at various amount of chitosan added.

Sample	mass of chitosan added (g)	Pb^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.20	0.15	0.01	0.08	0.10
2	0.50	0.23	0.14	0.19	0.10
3	1.00	0.22	0.12	0.17	0.10
4	1.50	0.18	0.12	0.15	0.10
5	2.00	0.23	0.11	0.17	0.10

4.4 Optimum of Metal Ion Removed with Time Effect

The results for zinc, iron and lead ion removed by chitosan based on contact time effect as shown in Table 4.6, Table 4.7 and Table 4.8 accordingly.

Table 4.6: Results of Zn^{2+} ion uptake after the addition of 2.00g of chitosan at different time in contact.

Sample	time in contact (hr)	Zn^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.30	2.69	2.54	2.62	1.00
2	2.00	2.71	2.57	2.64	1.00
3	4.00	2.71	2.57	2.64	1.00
4	6.00	2.72	2.58	2.65	1.00
5	8.00	2.68	2.55	2.62	1.00

Table 4.7: Results of Fe^{2+} ion uptake after the addition of 2.00g of chitosan at different time in contact.

Sample	time in contact (hr)	Fe^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.30	11.05	11.23	11.14	1.00
2	2.00	12.29	12.48	12.39	1.00
3	4.00	13.09	13.44	13.27	1.00
4	6.00	13.27	13.35	13.31	1.00
5	8.00	14.62	14.63	14.63	1.00

Table 4.8: Results of Pb^{2+} ion uptake after the addition of 2.00g of chitosan in waste water at different time in contact.

Sample	time in contact (hr)	Pb^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	0.30	0.23	0.16	0.20	0.10
2	2.00	0.07	0.07	0.07	0.10
3	4.00	0.23	0.16	0.20	0.10
4	6.00	0.17	0.11	0.14	0.10
5	8.00	0.23	0.15	0.19	0.10

4.5 Optimum of Metal Ion Removed with Temperature Effect

The results for zinc, iron and lead ion removed by chitosan based on temperatures effect as shown in Table 4.9, Table 4.10 and Table 4.11.

Table 4.9: Results of Zn^{2+} ion uptake after the addition of 2.00g of chitosan in waste water at various temperature conditions.

Sample	Temperature (°C)	Zn^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	25	2.71	2.57	2.64	1.00
2	40	2.59	2.47	2.53	1.00
3	50	2.60	2.51	2.56	1.00
4	60	2.69	2.55	2.62	1.00
5	70	2.74	2.59	2.67	1.00

Table 4.10: Results of Fe^{2+} ion uptake after the addition of 2.00g of chitosan in waste water at various temperature conditions.

Sample	Temperature (°C)	Fe^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	25	13.31	13.46	13.39	1.00
2	40	9.16	9.19	9.18	1.00
3	50	11.01	10.80	10.91	1.00
4	60	10.79	10.71	10.75	1.00
5	70	13.52	13.30	13.41	1.00

Table 4.11: Results of Pb^{2+} ion uptake after the addition of 2.00g of chitosan in waste water at various temperature conditions.

Sample	Temperature (°C)	Pb^{2+} ion uptake (ppm)		Average	EQA 1974 of Malaysia Std (Sewage & Industrial Effluents) mg/l
		1st Test	2nd Test		
1	25	0.23	0.14	0.19	0.10
2	40	0.23	0.14	0.19	0.10
3	50	0.20	0.12	0.16	0.10
4	60	0.23	0.14	0.19	0.10
5	70	0.23	0.14	0.19	0.10

CHAPTER 5

DISCUSSION

5.1 The Total Amount of Chitosan Extracted From Crab Shells

The result of the total mass obtained during the extraction of chitin until the production of chitosan is presented in Table 4.1.

The yield of chitin from the extraction step which involved the demineralization and deproteination processes, composed together with indeterminate amount of impurities which is about 42.83g. This impurities are in a formed of coarse powder that believed to be the un-reacted crab shells during the demineralization and deproteination processes. The yield of chitosan from the deacetylation process of chitin was **67.95 weight %**.

This percentage value was largely different from the previous final year research project. Based on the previous research project reported, only **46.67 weight %** of chitosan yielded from cockroaches' exoskeleton. It is also mentioned that the weight lost of chitosan was due to human errors. However, in this project, the chitosan lost was due to some errors incurred during the filtration stages.

The mass of chitosan lost was incurred when there were some of the filter papers had accidentally shredded while conducting the filtration process. This was due to over pressure of air suction in the conical flask. Besides that, the mass of the filtrated and dried products were lost since some of the residue could not be removed due to a strong bonding between the residues and filter paper. Thus, this are some of the causes that contribute to the lost of powder residues. Other than that, it was all involved human errors while handling the experimental and measuring works.

During the extraction of chitosan, there was some behavior observed as each part of the process was carried out. In the first process, where by a demineralization process were taken placed, a formation of foams were observed as 20ml of 1.5M of HCl added in each gram of crab shells powder. The foam was slightly decreasing in continuous stirring. An unpleasant odor was smelled as the reaction occurred. This is believed to be the production of calcium salt, $CaCl_2$ and CO_2 released as the mixture counteract to each other.

In the deproteination process, a white solution was formed as 20ml of 1.2M of NaOH was added for every gram of shells powder added. After a length of time, there were white precipitates observed settling at the bottom surface of the beaker (Figure 3.2). The mixture seems to be heterogeneously mixed, since the powder added was not dissolving after undergoing certain of processes. According to Khor [16] mentioned that chitosan are not normally water soluble (with exception of low molecular weight components) at the body's pH 7 but can be subject to effects of erosion caused by constant interaction with bodily fluids for example.

5.2 The Initial Concentration of the Selected Metals Ion Present in Industrial Waste Water

Table 4.2 shows the initial concentration of metal ion present in the waste water sample collected from FELCRA Nasaruddin Palm Oil Mill. From the result shown, it can be observed that the metals ion that present in the untreated waste water gave the highest concentrations number where 24.56 ppm of iron followed by 2.83 ppm of zinc and 0.23 ppm of lead ion were detected. Therefore, the top three metals ion which are Fe^{2+}, Zn^{2+} and Pb^{2+} ions were selected to be studied in this research project.

5.3 The Optimum Amount of Chitosan in Metal Ions Removal

In order to determine the optimum amount of chitosan required to remove the selected metal ion, each of 30ml of waste water sample has been added with different mass of chitosan powder which were 0.20g, 0.50g, 1.00g, 1.50g and 2.00g. The samples have been left 12.00 hours with continuous stirring for reaction to occur.

Thus, by referring to Figure 5.1, Figure 5.2, and Figure 5.3, it can be observed that the metal ion reduction in the waste water is proportionally increases as the amount of chitosan added was increased. The results of the FAAS analysis shows that as

2.00 g of chitosan added, the metal ion reduced to 69.81 wt % of Fe^{2+}, followed by 73.91 wt % of Pb^{2+} and 94.17 wt % of Zn^{2+} from waste water. The extracted chitosan, shows the highest performance in removing zinc ion, followed by lead and iron ions.

This is means that more metal ion can be removed with the large amount of chitosan. It has been proven by theoretically, where the increase dosage of polymer (chitosan) amount added, increased the amount of metal ion removed by all biopolymers independently of the temperature and contact time used [14] *(Refer Appendix 1)*

Therefore, the overall optimum amount of the chitosan that will be used in this experiment is 2.00g. This amount was applied for the study of metal ion removal at various times in contact and temperatures.

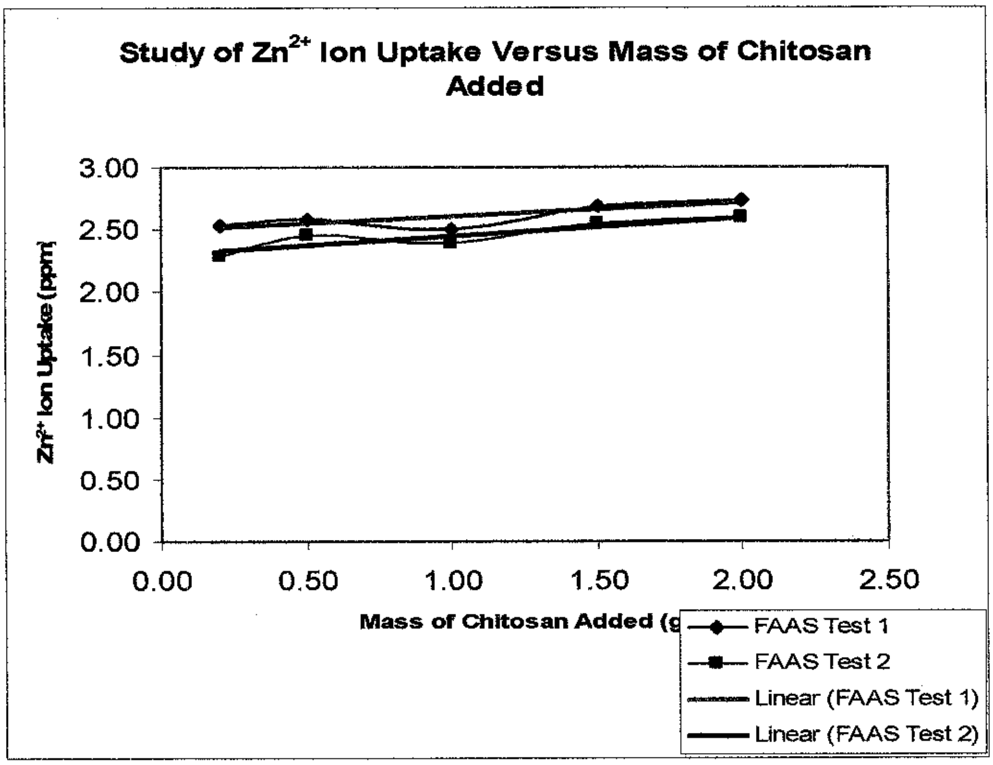


Figure 5.1: Study of Zn^{2+} ions uptake versus mass of chitosan added.

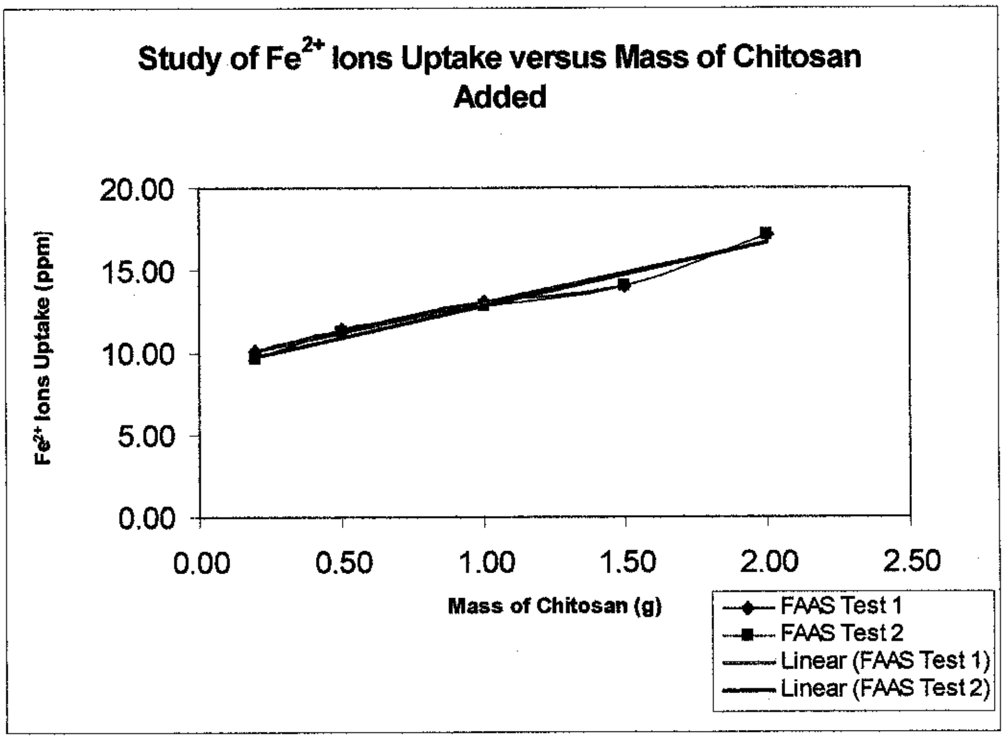


Figure 5.2: Study of Fe^{2+} ions uptake versus mass of chitosan added.

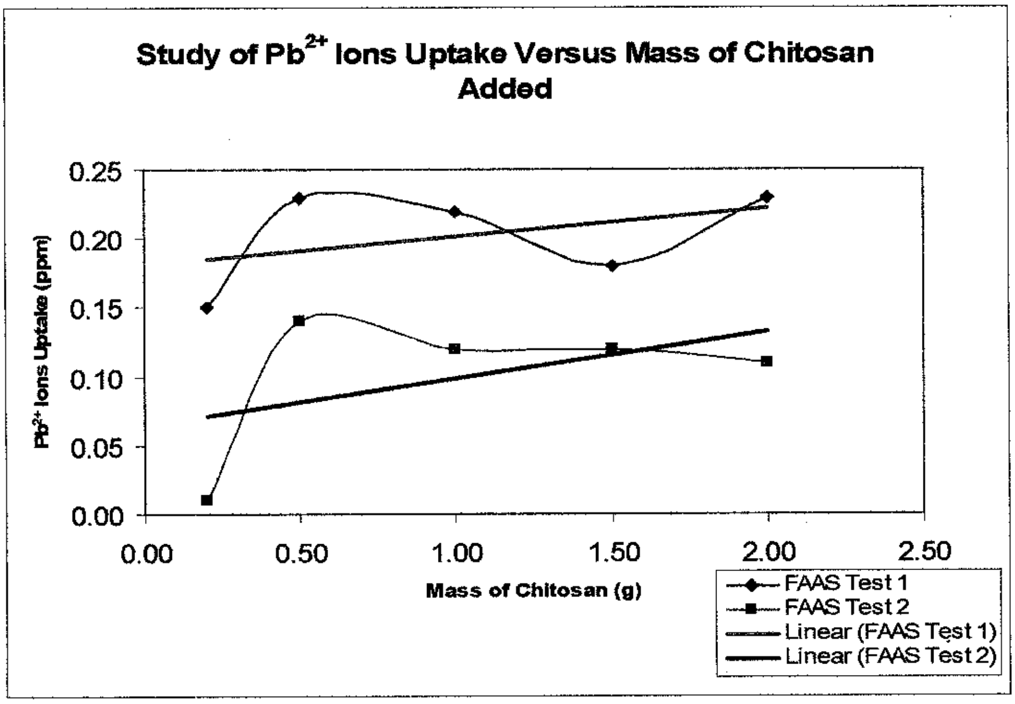


Figure 5.3: Study of Pb^{2+} ions uptake versus mass of chitosan added.

5.4 Efficiency of Metal Ion Removal at Different Time in Contact

In this experiment, 2.00g chitosan was added in 30ml of the industrial waste water collected. Five set of samples have been prepared in order to study on the efficiency of metal ion removal at different time in contact. Each of the samples was let to react under continuous stirring at 5 different time intervals, which were at 0.30, 2.00, 4.00, 5.00, 6.00 and 8.00 hours. Finally, these samples were filtrated to remove the reacted chitosan residue before conducting the FAAS test method.

By referring to the results shown; Figure 5.4, Figure 5.5 and Figure 5.6, the percentage of metal ion removed at time interval 0.30 hours are calculated to be 40.19 wt %, 34.78 wt % and 92.40 wt % of Fe^{2+}, Pb^{2+} and Zn^{2+} ions respectively. These percentages of metal ion reduction have been increased as the time interval was raised to 8.00 hours. However, for metal ion reduction of zinc ion was seems to be not affected by the time interval. This is because the amount of zinc ion is more or less removed into completion with 2.00 g of chitosan added.

The trend of the graph is comparable with the research conducted by K.M. Peiselt da Silva and M.I. Pais da Silva [14] where from the study; it has shown that, the copper ion removal is proportional to the time in contact. *(Refer Appendix 1).*

The results for final percentage of metal ion removed in this experiment at 8.00 hours of time interval is 69.81 wt % for iron, 73.91 wt % for lead and 92.40 wt % for zinc ions.

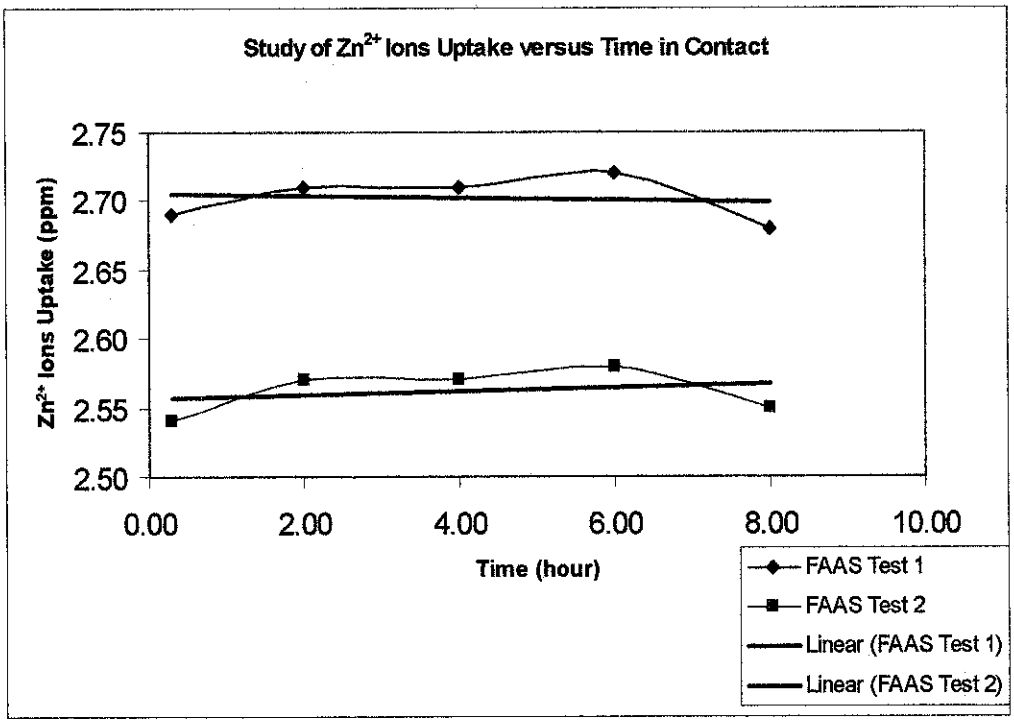


Figure 5.4: Study of Zn^{2+} ions uptake versus time in contact ($m_{chitosan} = 2.00g$).

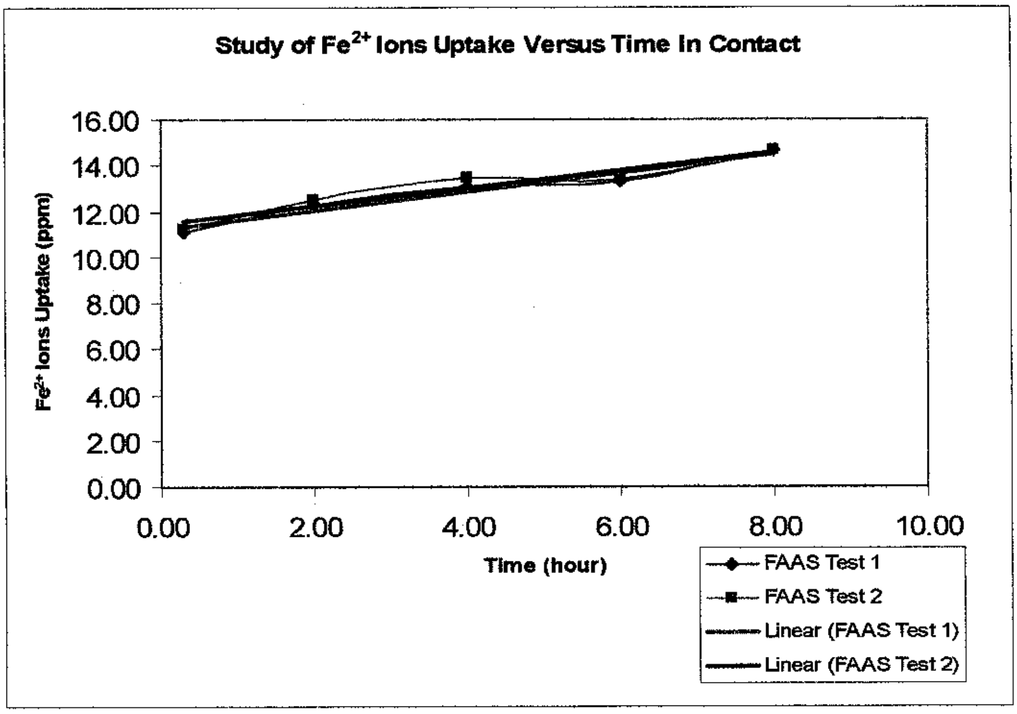


Figure 5.5: Study of Fe $^{2+}$ ions uptake versus time in contact ($m_{chitosan} = 2.00g$).

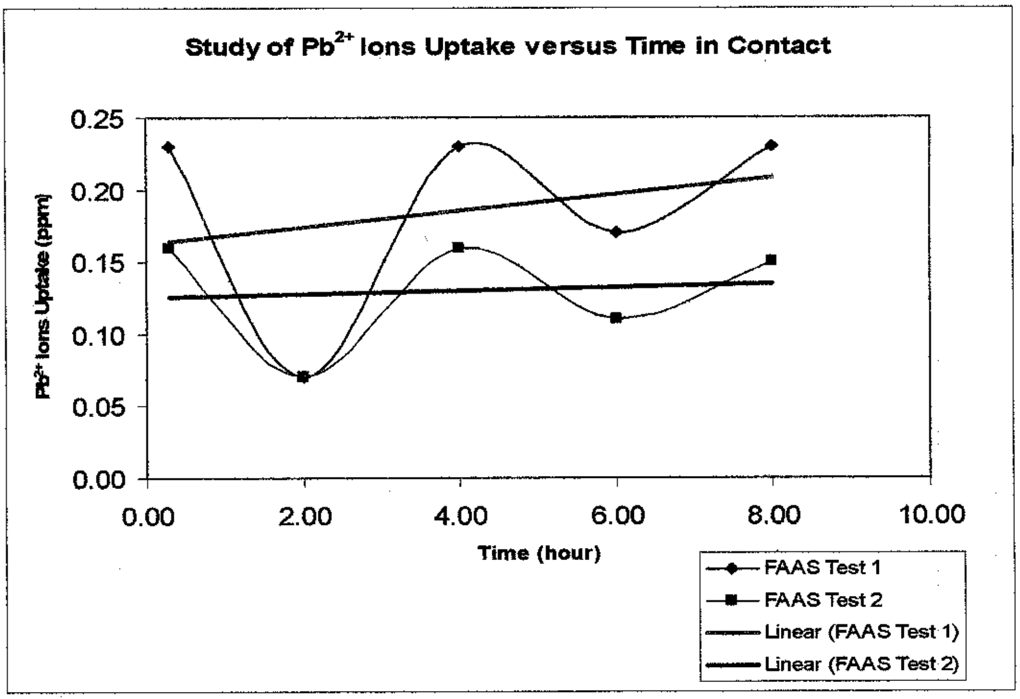


Figure 5.6: Study of Pb $^{2+}$ Ions Uptake versus Time in Contact ($m_{chitosan}$ = 2.00g).

5.5 Efficiency of Metal Ions Removal at Various Temperature Conditions

In this experiment, 2.00 g of chitosan was added in 30ml of industrial waste water. Similarly as in the previous experiment, five set of samples were prepared and let to react under continuous stirring for 30 minutes. Further reactions were taken placed in an oven where by, each of the samples was heated at various temperature conditions. The experiment was conducted with the reduction of metal ion by chitosan at 40°C, 50°C, 60°C, 70°C and at ambient temperature conditions.

From the results show in Figure 5.7, Figure 5.8 and Figure 5.9, it can be observed that the data of metal ion removal in the waste water sample are seems to be fluctuating over different temperature conditions. By referring to the trend line plotted, the ability of the metal ion reduction is not affected by the temperature conditions.

This shows that the efficiency of the metal ion reduction by chitosan is uncorrelated with the temperature conditions. This is proven by the research conducted by K.M.

Peiselt da Silva and M.I. Pais da Silva [14]. Figure 1 *(Appendix 2)* shows that the increment of temperature from 25°C to 75°C improved the copper retention but a further increasing to 100°C did not enhance the removal significantly. It seems that the chitosan starts to be degraded.

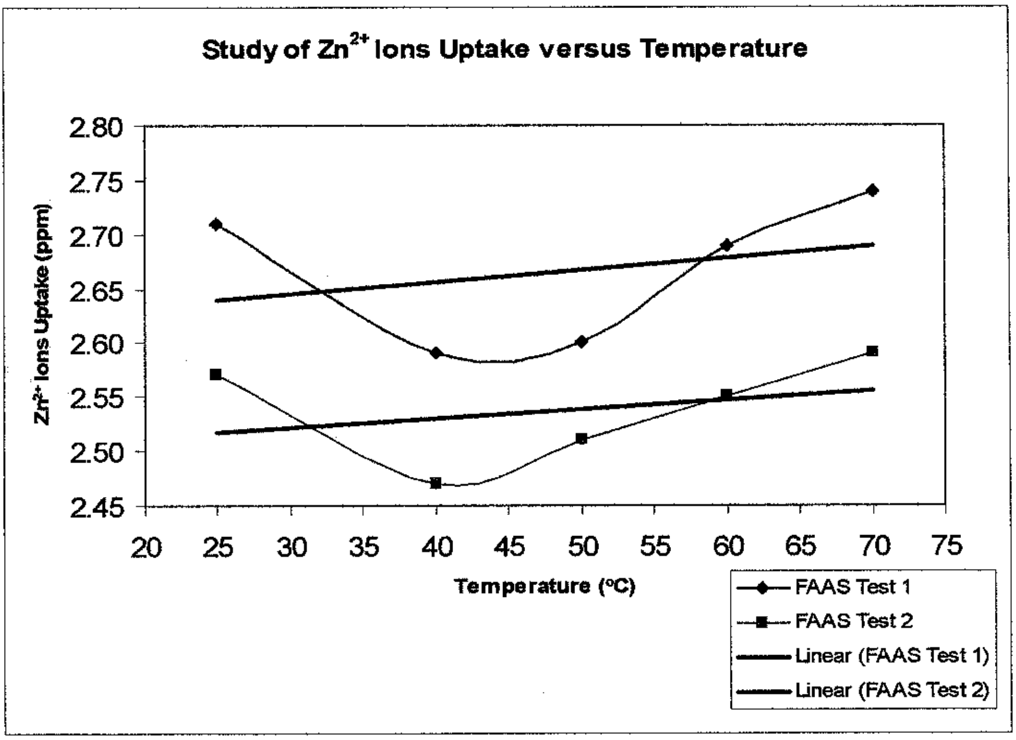


Figure 5.7: Study of Zn^{2+} ions uptake versus temperature ($m_{chitosan} = 2.00g$).

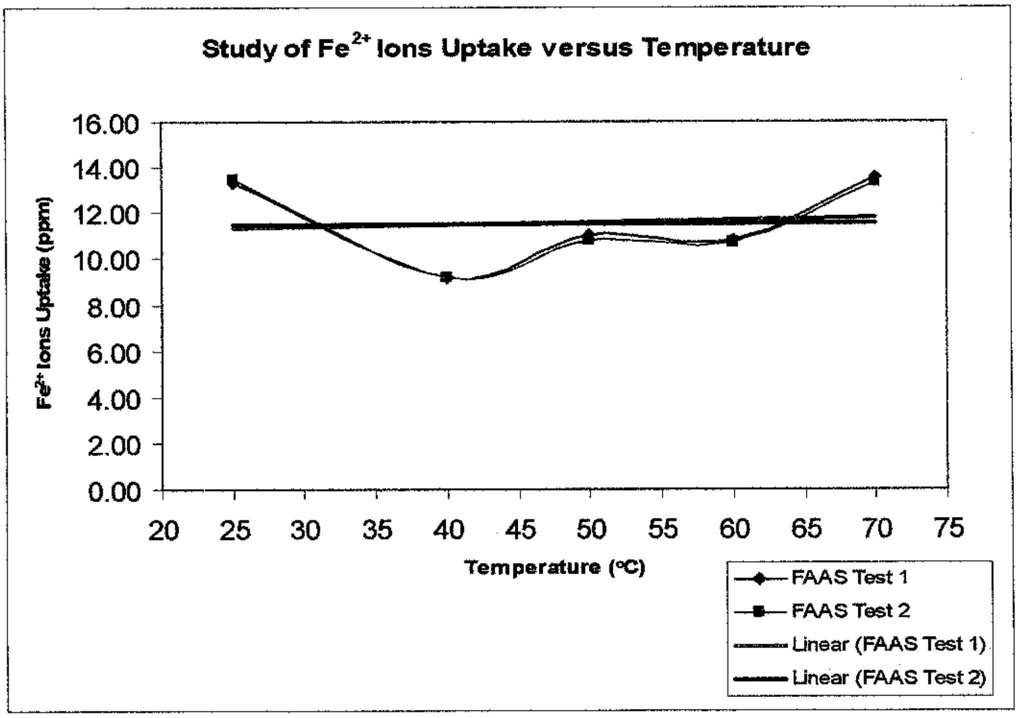


Figure 5.8: Study of Fe $^{2+}$ ions uptake versus temperature ($m_{chitosan} = 2.00g$).

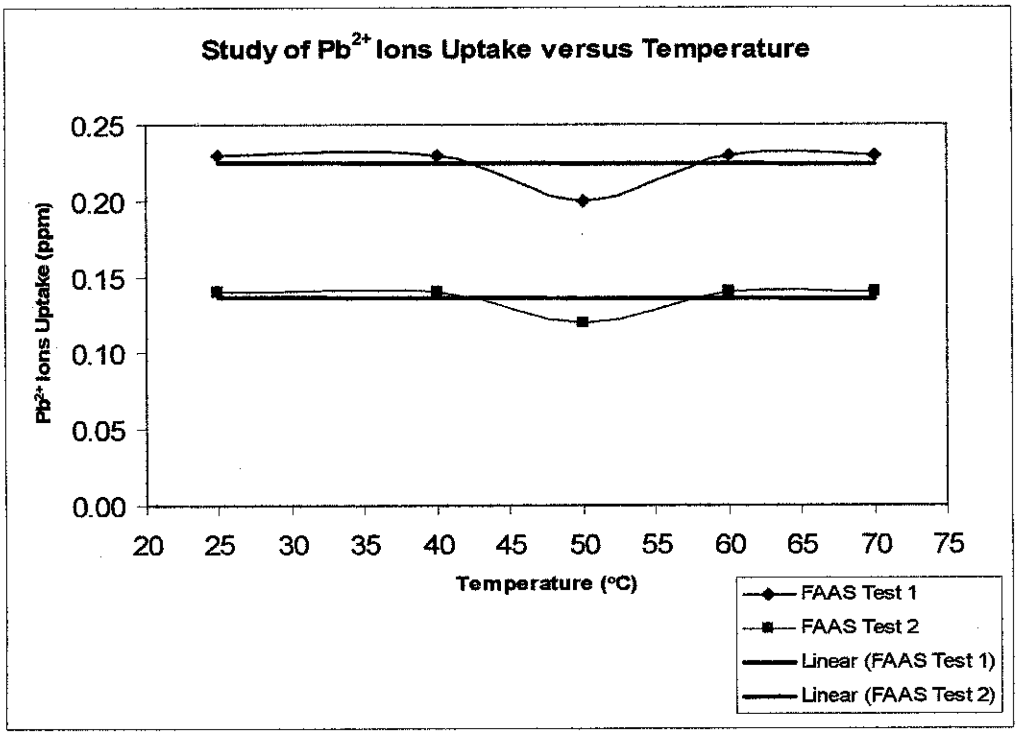


Figure 5.9: Study of Pb^{2+} ions uptake versus temperature ($m_{chitosan} = 2.00g$).

5.6 Summary of Heavy Metal Ion Removal

The percentage of metal ion removed for zinc, iron and lead ion by using different dosage of chitosan is shown by Figure 5.10. The highest percent removal recorded is zinc ion at 94.17% with treatment of 2.00g of chitosan followed by lead ion at 80.43% with 0.50g chitosan and for iron at 69.81% with 2.00g of chitosan.

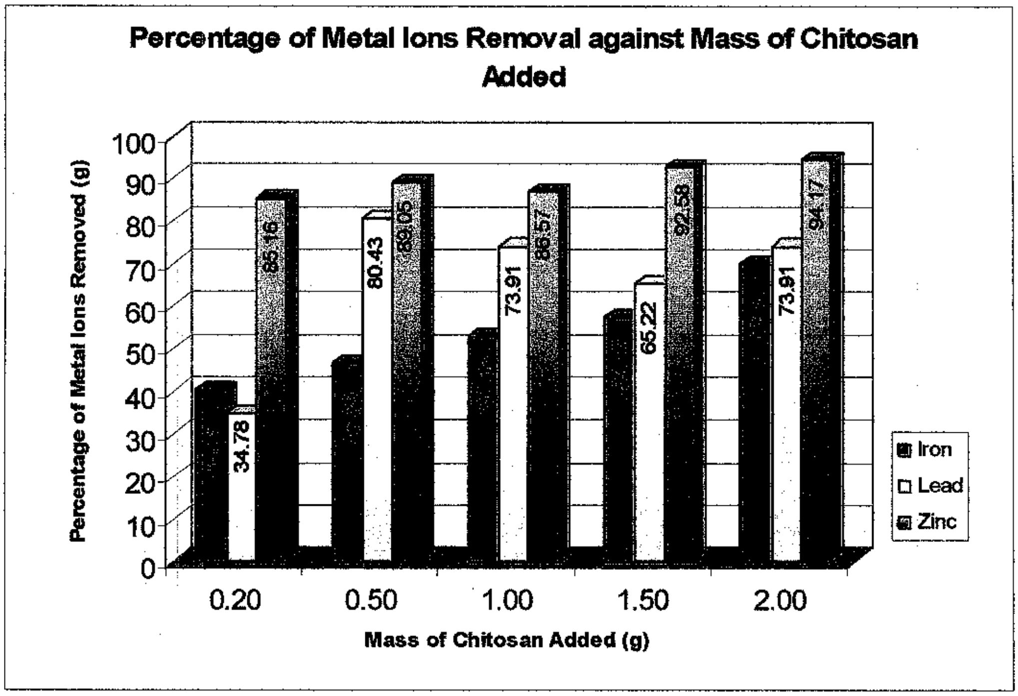


Figure 5.10: Percentage of metal ions removal against mass of chitosan added

From the optimum dosage for overall metal ions removal ($m_{chitosan} = 2.00g$), two parameters effect which are the contact time (Figure 5.11) and temperatures (Figure 5.12) were studied.

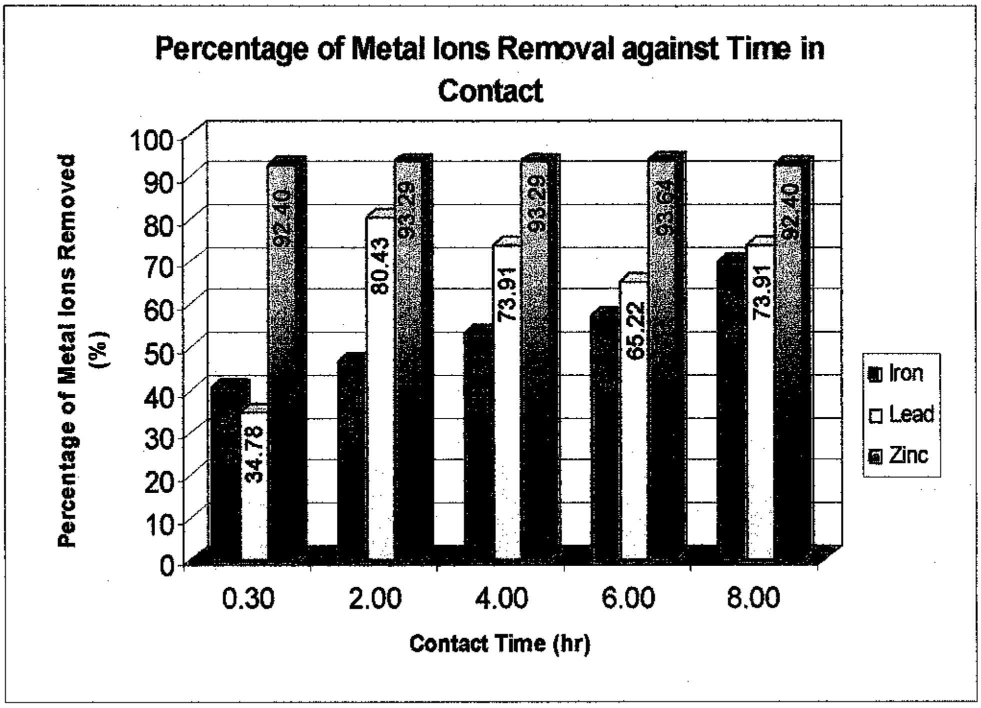


Figure 5.11: Percentage of metal ions removal against time in contact ($m_{chitosan}$ = 2.00g).

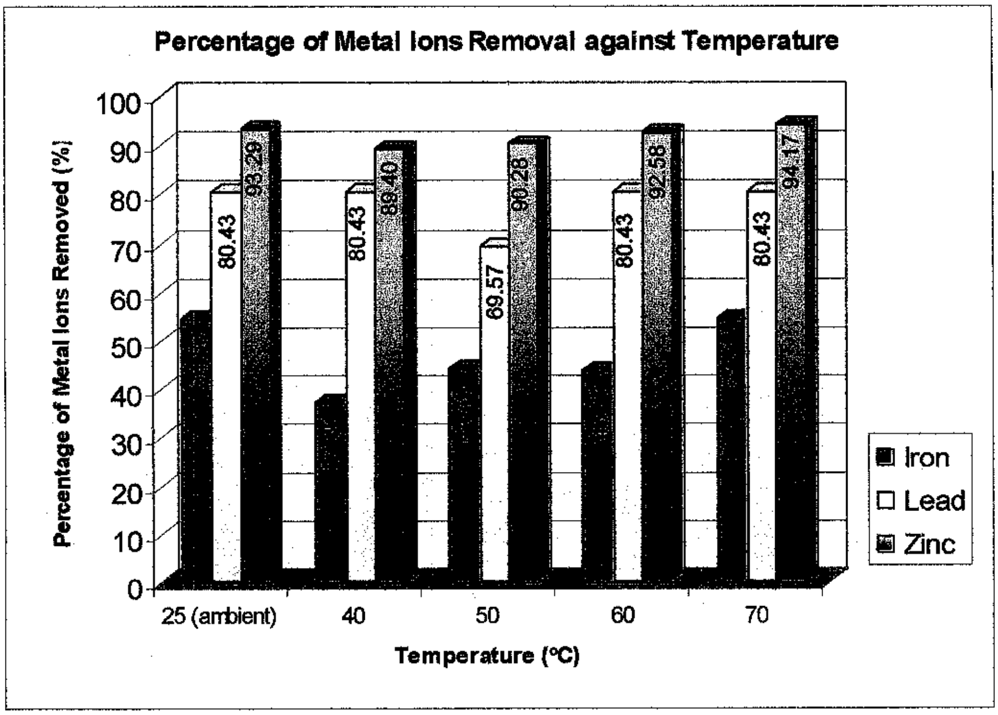


Figure 5.12: Percentage of metal ions removal against temperature ($m_{chitosan}$ = 2.00g).

From the result shown in Figure 5.11 and Figure 5.12, contact time does not gives any significant different to the percentage removal of the three metal ion similarly to the temperature effect. This might be due to the dosage of chitosan ($m_{chitosan} = 2.00g$) that were used in this experiment has optimally removed the metal ion.

CHAPTER 6
CONCLUSION

In the first part of the objective which were to extract chitosan from crab shell and determine the weight percentage of biopolymer that can be extorted, was achieved when 67.95 wt % which equivalent to 30.32g of chitosan powder yield from 100g of crab shells powder that were used in the extraction process. Theoretically, the optimum amount of chitosan that can be extracted from the source of chitin is 70%. Therefore, this extraction method is precisely meeting the target.

As the first part of the objective has been achieved, this experiment has moved on to the second objective which is to study on the efficiency of metal ion removal in industrial waste water by chitosan. The highest percent removal recorded is zinc ion at 94.17% with treatment of 2.00g of chitosan followed by lead ion at 80.43% with 0.50g chitosan and for iron at 69.81% with 2.00g of chitosan added.

From the study conducted, the efficiency of the metal ion reduction in the waste water is proportionally increased with the amount of chitosan added. Similarly, the data showed from the metal ion reduction against the time interval gave the same trend of results. However, the metal ion reduction independent to the temperatures and contact times effect.

The Final Year Research Project of Semester July 2004 has been completed within the time frame given and most of the above results have achieved the objectives of this research project.

CHAPTER 7

RECOMMENDATIONS AND FURTHER STUDIES

Even though the project has met its target, but there are some possibilities of error that might involved while doing the experiment. These potential errors are due to human and equipment errors. However, the occurrence of these errors can be reduced by taking the pre-cautions steps while handling the experiments.

There are some recommendations that can be applied in order to increase the effectiveness of the study. These recommendations are stated as below:

i. Further study on the content of extracted crab shell to chitosan should be conducted in order to determine the exact amount of the biopolymer. This can be conducted by using chemical and X-ray Fluorescene analysis of chitosan sample. In theory, ash, silica, calcium, chloride and nitrogen have been found to be existing in chitosan.

ii. Instead of that, this study will be more interesting if it was conducted at different grain size of chitosan. Thus, the effect of grain size towards the metal ion removal can be analyzed.

iii. Since in this experiment, the percentage of metal ion reduction by chitosan has been identified, thus it is recommended that a further study on metal ion removal by using chitin and modified chitosan shall be conducted in the future. From this, the effectiveness of the biopolymers can be analyzed and compared.

iv. Finally, the experimental procedures must be consistent for all the analysis that will be conducted. Therefore, the conditions of equipments and apparatus has to be ensured before conducting the experiment

REFERENCES

1. R. A. A. Muzzarelli, Chitin, Pergamon Press, New York, 1977.

2. N.K. Mathur and C.K. Narang. *J. Chem. Educ.* **67** (1990), pp. 938-942.

3. P.R. Austin, C.J. Brine, J.E. Castle and J.P. Zikakis. *Science* **212** (1981), pp. 149 – 753. Abstract – MEDLINE.

4. R.L. Rawis. *Chem. Eng. News* **14** (1984), pp. 42 – 45.

5. E. Longhinlotti, L. Furlan, M.C.M. Laranjeira and V.T. Favera. Quinn. Nova **19** (1996), pp. 221 – 224.

6. R.A.A. Muzarelli and O. Tubertini, Talanta **16** (1969), pp. 1571 – 1577. Abstract.

7. L. Lepri, P.G. Desideri and G. Tanturli. *J. Chromatogr.* 147 (1978), pp. 375 – 381. Abstract.

8. A. Valentini, M.C.M. Larenjeira, S. Fiori an V. T. Favera. *Quinn. Nova* **23** (2000), pp. 12-15.

9. O. Gyliene, R. Rekertas and M. Salsukas. *Water Res.* 36 (2002), pp. 4128 – 4136.

10. B. Benguella and H. Benaissa. *Colloids Surf. A: Physicoshem. Eng. Aspects* **201** (2002), pp. 143 – 150.

11. K. H. Chu. *J. Hazard. Mater.* **90** 1 (2002), pp. 77 – 95.

12. V. Sycha, I. Lang and G. Sebar. *Prog. Anal. At. Spectrosc.* **4** (1981), pp. 341 – 346

13. W. W. Eliot, C. Heatheote and R. A. Mostyn. *Talanta* **19** (1072), pp. 359 – 363.

14. K.M. Peiselt da Silva and M.I. Pais da Silva . *Physicochemical and Engineering Aspect.* **237** (2004), pp. 15-21.

15. Alimunar, A. and R. Zainuddin. (1992), An Economical Technique for Producing Chitosan, In Brine et al, (1992).

16. Khor, Eugene. 2001, Chtitin: Fulfilling a Biomaterials Promise, Elseiver Science Ltd, Oxford UK.

17. http://1dambies.free.fr/chitosan.html

18. http://www.sciencedirect.com/sciece

19. http://chitosan@dalwoo.com

20. http://www.ce.vt.edu/programs_area/environment

APPENDICES

RAW DATA

Appendix 1

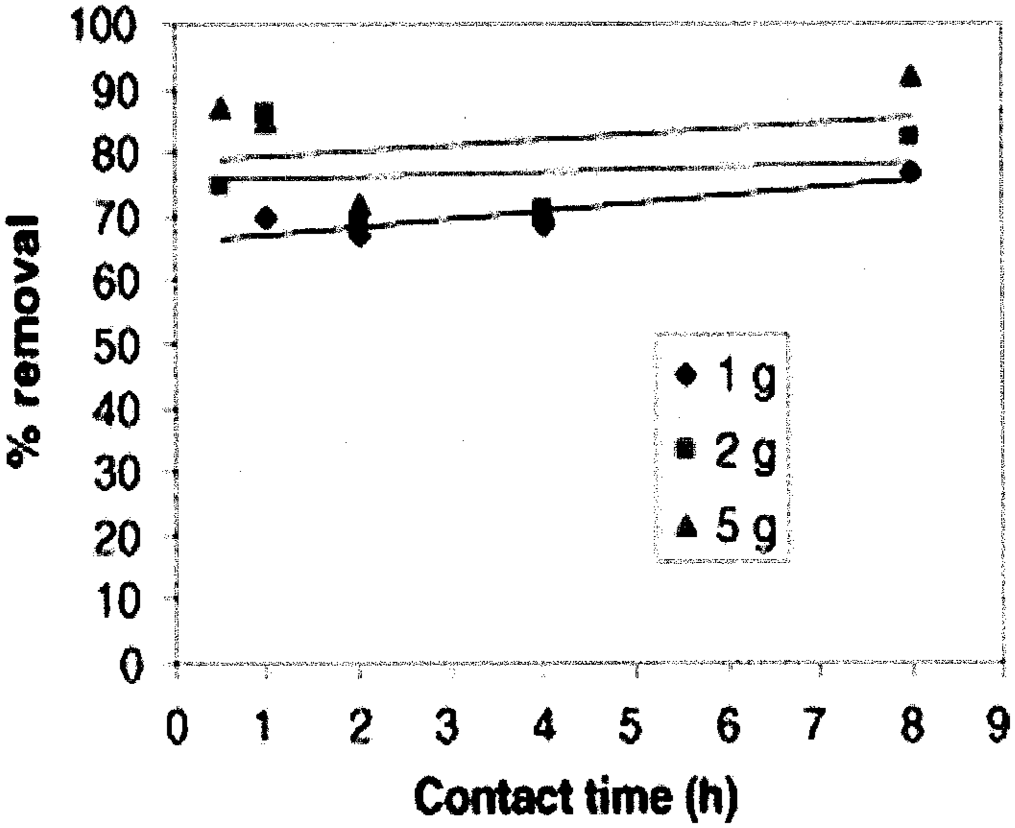


Figure 1: Influence of polymer amount on copper removal by chitosan (grain size, 35–60 mesh; *T*, 50°C).

Appendix 2

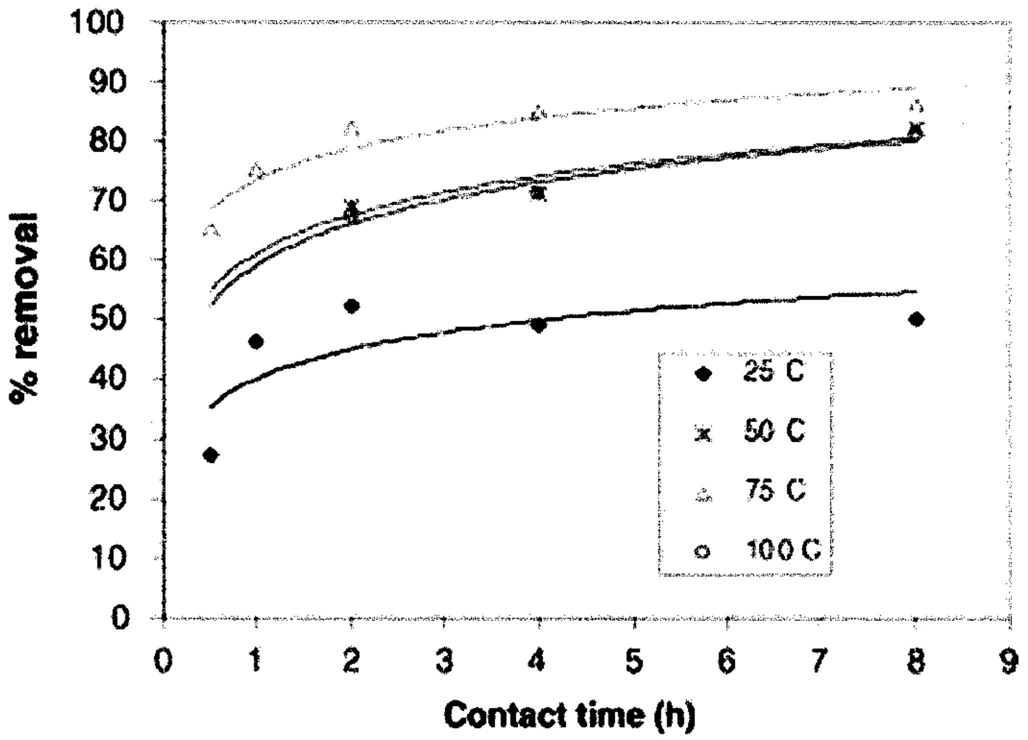


Figure 2: Influence of temperature on copper removal by chitosan (m polymer, 2 g; grain size, 35–60 mesh).

Appendix 3

Table 3: Results of metal ion leftover after the addition of different mass of chitosan in industrial waste water.

Mass of chitosan added (g)	Concentrations (ppm)		
	Zn	Fe	Pb
0.20	0.29	14.44	0.08
0.50	0.25	13.12	0.00
1.00	0.33	11.48	0.01
1.50	0.14	10.49	0.05
2.00	0.09	7.38	0.00

Table 4: Results of metal ion leftover after the addition of 2.00g of chitosan in waste water at different time of reaction

Time in contact (hr)	Concentrations (ppm)		
	Zn	Fe	Pb
0.30	0.14	13.51	0.00
2.00	0.12	12.27	0.16
4.00	0.12	11.47	0.00
6.00	0.11	11.29	0.06
8.00	0.15	9.94	0.00

Table 5: Results of metal ion leftover after the addition of 2.00g of chitosan in waste waste water at various temperature conditions.

Temperature conditions (°C)	Concentrations (ppm)		
	Zn	Fe	Pb
25.00	0.12	11.25	0.00
40.00	0.24	15.40	0.00
50.00	0.23	13.55	0.03
60.00	0.14	13.77	0.00
70.00	0.09	11.04	0.00